

# codex alimentarius commission



FOOD AND AGRICULTURE  
ORGANIZATION  
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WORLD  
HEALTH  
ORGANIZATION



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CX 4/40.2

**CL 2005/52 - PR**  
**December 2005**

TO: Codex Contact Points  
Interested International Organizations

FROM: Secretary, Joint FAO/WHO Food Standards Programme  
FAO, Viale delle Terme di Caracalla, 00100 Rome, Italy

SUBJECT: **REQUEST FOR COMMENTS ON:**

**Proposed Draft Revision of the List of Methods for Pesticide Residue Analysis at Step 3**

DEADLINE: **1 March 2006**

COMMENTS:	<b>To:</b> Dr Piet VAN ZOONEN National Institute of Public Health and the Environment PO Box 1 3720 BA Bilthoven The Netherlands Fax: +31 30 274 4424 E-mail: <a href="mailto:piet.van.zoonen@rivm.nl">piet.van.zoonen@rivm.nl</a>	<b>Copy to:</b> Secretary Joint FAO/WHO Food Standards Programme FAO Viale delle Terme di Caracalla 00100 Rome, Italy Fax: +39 06 5705 4593 E-mail: <a href="mailto:codex@fao.org">codex@fao.org</a>
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## BACKGROUND

1. From the responses on CL 1998/30-PR<sup>1</sup> it became clear that the majority of the laboratories use modifications of methods published in either one of the following manuals: Official Methods of AOAC INTERNATIONAL; Pesticide Analytical Manual, Food and Drug Administration, USA; Manual of Pesticide Residue Analysis, Deutsche Forschungsgemeinschaft (German or English edition); or Analytical Methods for Residues of Pesticides Inspectorate for Health Protection of the Netherlands. The majority of the responses referred to pesticides amenable to gas chromatography or the analysis of carbamates by liquid chromatography with fluorescence detection. These methods cover approximately 75% of the compounds in the Codex system.

2. In CL 2002/16-PR requested Member governments and interested organizations to provide descriptions of their analytical methods together with their scope and available validation data. In previous

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<sup>1</sup> CX/PR 99/10

discussions it was stressed that methods included in the list should reflect current rather than past practices in pesticide residue analysis.

3. The responses to CL 2002/16-PR yielded more recent information on validated methods that are currently in use<sup>2</sup>. Moreover in its 35<sup>th</sup> session the Committee was informed by the Delegation of Germany on a new LC/MS multi-method that covers many pesticides that were not covered by multi-residue methods before<sup>3</sup>. The Committee decided that older references for these compounds are to be deleted. The Delegation of the Netherlands offered to review the list of methods and to identify the pesticides for which MRLs have been set but for which no suitable methods were made available to the Committee during the last 5 years.

#### SUMMARY OF METHODS AVAILABLE TO THE COMMITTEE AT PRESENT

4. The current Circular Letter provides an inventory of available (see Annex I) and submitted methods. The letter also incorporates a question on the analysis of individual dithiocarbates, possible false positive signals might emerge from the methods based on the conversion into carbondisulphide.

5. Germany submitted a number of European standardized methods and provided information on their on their scope, principle validation data and further aspects, where appropriate<sup>4</sup>. The methods submitted cover both pesticide residues as well as contaminants. Reference to the methods submitted by Germany is given in Table 1.

**Table 1: European standardized methods for pesticide residue analysis**

EN 1528-1: 1996-10 (confirmed 2001)	Fatty food - Determination of pesticides and polychlorinated biphenyls (PCBs) - Part 1: General considerations	Type III
EN 1528-2: 1996-10 (confirmed 2001)	Fatty food - Determination of pesticides and polychlorinated biphenyls (PCBs) - Part 2: Extraction of fat, pesticides and PCBs and determination of fat content	Type III
EN 1528-3: 1996-10 (confirmed 2001)	Fatty food – Determination of pesticides and polychlorinated biphenyls (PCBs) - Part 3: Clean-up methods	Type III
EN 1528-4: 1996-10 (confirmed 2001)	Fatty food – Determination of pesticides and polychlorinated biphenyls (PCBs) - Part 4: Determination, confirmatory tests, Miscellaneous	Type III
EN 12393-1:1998-10	Non fatty food - Multiresidue methods for the gas chromatographic determination of pesticide residues – Part 1: General considerations	
EN 12393-2:1998-10	Non fatty food - Multiresidue methods for the gas chromatographic determination of pesticide residues – Part 2: Methods for extraction and clean-up	
EN 12393-3:1998-10	Non fatty food - Multiresidue methods for the gas chromatographic determination of pesticide residues – Part 3: Determination and confirmatory tests	
EN 12396-1:1998-10	Non fatty food - Determination of dithiocarbamate and thiuram disulfide residues - Part 1: Spectrometric method	
EN 12396-2:1998-10	Non fatty food - Determination of dithiocarbamate and thiuram disulfide residues - Part 2: Gaschromatographic method	
EN 12396-3:2000-05	Non fatty food – Determination of dithiocarbamate and thiuram disulfide residues - Part 3: UV-spectrometric xanthogenate method	
EN 13191-1:2000-04	Non fatty food - Determination of bromide residues Part 1: Determination of total bromide as inorganic bromide	
EN 13191-2:2000-04	Non fatty food - Determination of bromide residues Part 2: Determination of bromide	

<sup>2</sup> CX/PR 03/10

<sup>3</sup> J. Klein and L. Alder; *J. Assoc.. Off. Anal. Int.* 86 (2003) 1015.

<sup>4</sup> 34<sup>th</sup> session of the CCPR, CRD5

**CANADA**

6. Canada submitted brief descriptions of 8 methods currently used in their country:

**1. DETERMINATION OF 265 PESTICIDES IN FRUIT & VEGETABLES WITH SOLID PHASE EXTRACTION CLEAN-UP AND GC/MSD AND HPLC FLUORESCENCE DETECTION**

7. A representative sample is blended with acetonitrile and sodium chloride and the layers are separated by centrifugation. An aliquot of the acetonitrile phase is concentrated, and cleaned up on an Envi-Carb SPE cartridge which is connected in series with an aminopropyl sep-pak. The pesticides are eluted from the cleanup column with acetonitrile : toluene 3:1. The eluant is concentrated and solvent exchanged to acetone. The sample is then split for analysis of the multiresidues by GC/MSD, and the carbamates by reverse phase HPLC with post-column derivitization and fluorescence detection.

**2. DETERMINATION OF AMITRAZ IN FOOD BY GC/MSD**

8. The sample matrix is digested under acidic conditions which serves to hydrolyze Amitraz and its metabolites to 2,4-Dimethylaniline (2,4-DMA). The matrix is then made basic and extracted with iso-octane. A portion of the extract is filtered, and the analyte is derivatized using Heptafluorobutyric Acid Anhydride, and concentrated. The instrumental analysis is performed by capillary gas-liquid chromatography with Mass Selective Detection.

**3. DETERMINATION OF BENOMYL IN APPLES BY HPLC-UV**

9. A representative sample is blended with ethyl acetate, filtered and concentrated. HCl is added and the acidified mixture is heated for one hour at 80°C to hydrolyze benomyl to carbendazim. After washing with hexane and ethyl acetate, the acidic aqueous phase is made basic by the addition of sodium carbonate solution. The resulting carbendazim is extracted with ethyl acetate and the ethyl acetate extract is evaporated. The residue is dissolved in methanol and passed through a Florisil Sep Pak cartridge. Analysis is performed by high pressure liquid chromatography with UV detection.

**4. DETERMINATION OF THIABENDAZOLE IN FRUITS AND VEGETABLES BY HPLC-UV AND HPLC-FLUORESCENCE**

10. A representative sample is blended with acetonitrile and sodium chloride (NaCl). The layers are allowed to separate. A portion of the acetonitrile phase is cleaned up using an aminopropyl solid phase extraction (SPE) cartridge. The eluent is concentrated and solvent-exchanged to the mobile phase. The quantitation is performed using HPLC/UV detection or fluorescence detection, where UV interferences are observed.

**5. DETERMINATION OF ETU (2-IMIDAZOLIDINETHIONE) IN FRUIT AND VEGETABLES BY GC/AED**

11. The sample matrix is extracted using methanol. The ETU is derivatized by the alkylation of the thiocarbonyl group to form Benzylthio-2-imidazoline using benzyl chloride. The matrix is made acidic and washed with dichloromethane, then made basic and the analyte is extracted using dichloromethane. The extract is concentrated and derivatized further using Trifluoroacetic Anhydride. The quantitation is performed by capillary gas-liquid chromatography with atomic emission detection (AED) using the sulphur channel.

**6. DETERMINATION OF ORGANOCHLORINATED PESTICIDES AND PCBs IN EGG AND DAIRY PRODUCTS BY GC/ECD**

12. The fat, containing the organochlorine pesticides, is extracted from the dairy sample matrix with hexane using a blender.

13. The egg sample matrix is extracted with dichloromethane using an chromatographic column.

14. The extracts are then purified using a Gel Permeation Chromatography (GPC) system, and

the quantitation is performed by capillary gas-liquid chromatography with electron capture detection.

## **7. DETERMINATION OF DAMINOZIDE IN APPLES BY GC-MSD**

15. Daminozide in apples is hydrolyzed in the presence of NaOH to form unsymmetrical dimethylhydrazine (UDMH). The generated UDMH is distilled from the matrix and it reacts with salicylaldehyde to form salicylaldehyde dimethyl hydrazone which is analyzed by gas chromatography using a mass selective detector.

## **8. DETERMINATION OF EBDC (ETHYLENE BIS-DITHIOCARBAMATES) IN FRUITS AND VEGETABLES BY HPLC WITH FLUORESCENCE DETECTION**

16. A representative sample is digested with hydrochloric acid and the resulting ethylenediamine is isolated with an ion exchange column, derivatized with o-phthalaldehyde (OPA) and determined by HPLC/fluorescence detection.

## **UNITED STATES OF AMERICA**

17. The United States of America submitted brief descriptions of the methods together with validation data utilized in their USDA Pesticide Data Program (PDP).

### **A. FRUIT AND VEGETABLES**

18. The USDA PDP laboratories are analyzing fresh and processed fruit and vegetable commodities using modifications of three multi-residue methods – the California Department of Food and Agriculture (CDFA) method, the Luke multi-residue procedure, and the New York Modified Solid-Phase Extraction (SPE) method. Each laboratory independently validates their modification of the method for the particular commodity/crop combinations analyzed by their facility.

19. *CDFA Multi-residue Method:* Adaptations of the multi-residue method developed by CDFA are used by four PDP laboratories – California, Washington, Florida/Tallahassee, and Florida/Winter Haven. For California and Washington, homogenized sample is extracted by blending with acetonitrile. Extracts are cleaned up using a C-18 SPE cartridge followed by a salting out step. Aliquots are then cleaned up according to the detection system employed – no clean-up for fractions analyzed via gas chromatography (GC)/flame photometric detection (FPD); florisil SPE clean-up for samples analyzed via GC/electron-capture detection (ECD), GC/micro-ECD, or GC/electrolytic-conductivity detection (ELCD); and aminopropyl SPE clean-up for fractions analyzed via high-performance liquid chromatography (HPLC) post-column derivatization systems, GC/mass spectrometry (MS), or LC/MS.

20. For Florida (Tallahassee and Winter Haven), homogenized sample is extracted by shaking with acetonitrile. Extracts are cleaned up using a C-18 SPE cartridge followed by a salting out step. Aliquots are then cleaned up according to the detection system employed - SAX/PSA SPE clean-up for samples analyzed via GC/FPD or GC/MSD; florisil SPE clean-up for samples analyzed via GC/halogen-specific detection (XSD); and aminopropyl SPE clean-up for fractions analyzed via HPLC post-column derivatization systems or LC/MS (Tallahassee only).

21. *Luke Multi-Residue Method:* Adaptations of the Luke multi-residue procedure are used by three PDP laboratories – Michigan, Ohio, and Texas. Homogenized sample is extracted by blending with acetone. The extract is filtered and pesticides partitioned from aqueous acetone to an organic phase via liquid-liquid extraction. Aliquots are then cleaned up according to the detection system employed and individual laboratory practice. In Ohio, analysis by GC/ELCD, GC/FPD, GC nitrogen-phosphorus detection (NPD), and GC/MSD requires no clean-up and carbamate analysis requires a simple solvent exchange. For Texas, analysis by GC/FPD requires no clean-up; GC/ELCD requires clean-up by florisil column; GC/MSD analysis requires a C-18 SPE clean-up; and carbamate analysis requires a simple solvent exchange. In Michigan, all fractions are solvent exchanged appropriate to the detection system used, except for LC/MS analysis, where a portion of each extract is cleaned up using an ENV SPE cartridge.

22. *New York Modified SPE Method:* This method is based on the Agriculture and Agri-Food Canada SPE method with some improvements based on the Luke extraction. It is applicable for extracting organochlorine, organophosphate, carbamate, and other pesticides from fruit, vegetables, and milk. For fruit and vegetables, homogenized sample is extracted by blending with 5% ethanol in acetonitrile. Extracts are salted out with sodium chloride, followed by sodium sulfate, and an aliquot cleaned up using SPE (Envicarb, SAX, and PSA). Portions of each extract are exchanged into appropriate solvents for analysis via GC (selective detectors and MS-MS) or LC (HPLC post-column derivatization for carbamates, LC/MS, or LC/MS-MS).

**PESICIDES FOR WHICH NO METHODS ARE AVAILABLE TO THE CCPR YET**

**23. Governments and International Organizations are invited to submit information on methods of analysis and their performance characteristics on the following pesticides:**

abamectine (177), amitraz (122), anilazine (163), azocyclotin (129), benalaxyl (155), benomyl (69), bentazone (172), bioresmethrin (93), bitertanol (144), buprofezin (173), cadusofos (174), cartap (97), chinomethionat (80), chlormequat (15), ciprodinil (207), clofentezine (165), cycloxydim (179), cyhexatin (67), cyromazine (169), dimethipin (151), diquat (31), dithianon (180), dodine (84), esfenvalerate (204), ethephon (106), ethoxyquin (35), etofenprox (184), etrimfos (123), famoxadone (208), fenbutatinoxide (109), fenproximate (193), fentin (40), fipronil (202), flusilazole (165), flutolanil (205), glufosinate-ammonium (175), glyphosate (158), guazatine (114), hexaconazole (170), hexathiazox (176), hydrogen phosphide (46), maleicx hydrazide (102), methacrifos (125), methoprene (147), methoxyfenozide (209), methylbromide (52), metiram (186), paclobutrazol (161), paraquat (57), penconazole (182), phentoate (128), 2-phenylphenol (56), pyraclostrobin (210), pyperinil butoxide (62), procloraz, (142), spinozad (203), thiophanate-methyl (77), trifloxystrobin (213) and triforine (116).

**24. Furthermore governments and international organizations are invited to give information on validated methods for the determination of individual dithiocarbamates.**

25. Descriptions of the methods will be submitted to IAEA publication on the IAEA Training and Research Centre (TRC) website.

## ANNEX I

## LIST OF PESTICIDES WITH REFERENCE TO THEIR METHODS OF ANALYSIS

177	Abamectine						
95	Acephate		EN 12393		CDFA		LCMS
117	Aldicarb				CDFA	PDP	LCMS
1	Aldrin and Dieldrin	EN 1528	EN 12393		CDFA	PDP	
134	Aminocarb						
122	Amitraz						
79	Amitrole						
163	Anilazine				CDFA		
68	Azinphos-ethyl		EN 12393				
2	Azinphos-methyl		EN 12393		CDFA	PDP	
129	Azocyclotin						
155	Benalaxyl						
137	Bendiocarb						LCMS*
69	Benomyl						
172	Bentazone						
178	Bifentrin		EN 12393		CDFA		
3	binapacril						
93	Bioresmethrin						
144	Bitertanol						
47	bromide ion			EN 13191			
4	Bromophos	EN 1528	EN 12393				
5	Bromophos-ethyl	EN 1528	EN 12393				
70	Bromopropylate		EN 12393				
173	Buprofezin						
139	Butocarboxim						
174	Cadusofos						
71	Camphechlor	EN 1528					
6	Captafol		EN 12393				
7	Captan		EN 12393		CDFA	PDP	
8	Carbaryl				CDFA	PDP	LCMS
72	Carbendazim						LCMS*
96	Carbofuran					PDP	LCMS*
9	Carbon disulphide						
10	Carbon tetrachloride						
11	Carbophenothion	EN 1528	EN 12393				
145	Carbosulfan						
97	Cartap						
80	Chinomethionat						
12	Chlordane	EN 1528			CDFA		
13	Chlordimeform						
14	Chlorfenvinphos	EN 1528	EN 12393				
15	Chlormequat						
16	Chlorobenzilate		EN 12393				
81	Chlorothalonil		EN 12393		CDFA		
201	Chlorpropopham		EN 12393				
17	Chlorpyrifos	EN 1528	EN 12393		CDFA		
90	Chlorpyrifos-methyl	EN 1528	EN 12393		CDFA	PDP	
207	Ciprodinil						
187	Clethodim						LCMS
156	Clofentezine						
18	Coumaphos		EN 12393				
19	Crufomate						
91	Cyanofenphos		EN 12393				
179	Cycloxydim						

157	Cyfluthrin	EN 12393	CDFA	
146	Cyhalothrin	EN 12393	CDFA	
67	Cyhexatin			
118	Cypermethrin	EN 12393		
207	Cyprodinil			LCMS*
169	Cyromazine			
104	Daminozide			LCMS*
20	2,4-D			LCMS*
21	DDT	EN 1528	EN 12393	CDFA PDP
135	Deltamethrin		EN 12393	CDFA
92	Demeton		EN 12393	LCMS
73	Demeton-s-methyl		EN 12393	LCMS
164	Demeton-s-Methylsulphon		EN 12393	LCMS
98	Dialofos		EN 12393	
22	Diazinon	EN 1528	EN 12393	CDFA
23	1,2-dibromoethane			
82	Dichlofluanid		EN 12393	
24	1,2-dichloroethane			
25	Dichlorvos	EN 1528	EN 12393	CDFA PDP
83	Dicloran		EN 12393	
26	Dicofol		EN 12393	CDFA
130	Diiflubenzuron			LCMS*
151	Dimethipin			
27	Dimethoate		EN12393	CDFA LCMS
87	Dinocap			
28	Dioxathion		EN 12393	
29	Diphenyl			
30	Diphenylamine			CDFA
31	Diquat			
74	Disulfoton		EN 12393	CDFA PDP
180	Dithianon			
105	Dithiocarbamates		EN 12396	
84	Dodine			
99	Edifenphos			
32	Endosulfan	EN 1528	EN 12393	CDFA
33	Endrin	EN 1528	EN 12393	
204	Esfenvalerate			
106	Ethephon			
107	Ethiofencarb			LCMS
34	Ethion	EN 1528	EN 12393	CDFA
149	Ethropophos		EN 12393	
35	Ethoxyquin			
108	Ethylenethiourea (ETU)			
184	Etofenprox			
123	Etrimfos			
208	Famoxadone			
85	Fenamiphos		EN 12393	CDFA PDP
192	Fenarimol		EN 12393	
109	Fenbutatin oxide			
36	Fenchlorphos	EN 1528	EN 12393	
37	Fenitrothion		EN 12393	PDP
185	Fenpropathrin		EN 12393	CDFA
188	Fenpropimorph			LCMS*
193	Fenproxymate			
38	Fensulfothion		EN 12393	

39	Fenthion	EN 1528	EN 12393	CDFA		
40	Fentin					
119	Fenvalerate		EN 12393	CDFA	PDP	
202	Fipronil					
152	Flucythrinate		EN 12393			
211	Fludioxonil					LCMS*
165	Flusilazole					
205	Flutolanil					
41	Folpet		EN 12393	CDFA		
42	Formothion		EN 12393			
175	Glufosinate-ammonium					
158	Glyphosate					
114	Guazatine					
194	Haloxypop					LCMS*
43	Heptachlor	EN 1528	EN 12393	CDFA	PDP	
44	Hexachlorobenzene	EN 1528	EN 12393			
170	Hexaconazole					
176	Hexathiazox					
45	Hydrogen cyanide					
46	Hydrogen phosphide					
110	Imazalil			CDFA	PDP	LCMS
206	Imidaclopride					LCMS
111	Iprodione		EN 12393	CDFA	PDP	
131	Isophenphos		EN 12393			
88	Leptophos					
48	Lindane	EN 1528	EN 12393	CDFA		
49	Malathion	EN 1528	EN 12393	CDFA	PDP	
102	Maleic hydrazide					
50	Mancozeb					
124	Mecarbam		EN 12393	CDFA		
138	Metalaxyl		EN 12393		PDP	LCMS
212	Metalaxyl-M					
125	Methacrifos					
100	Methamidophos		EN 12393	CDFA		LCMS
51	Methidathion		EN 12393	CDFA	PDP	
132	Methiocarb			CDFA		LCMS
94	Methomyl			CDFA	PDP	LCMS
147	Methoprene					
209	Methoxyfenozide					
52	Methyl bromide					
186	Metiram					
53	Mevinphos		EN 12393	CDFA		
54	Monocrotophos		EN 12393	CDFA	PDP	LCMS
181	Myclobutanil			CDFA		
140	Nitrofen		EN 12393			
55	Omethoate		EN 12393			LCMS
126	Ooxamyl			CDFA	PDP	LCMS
166	Oxydemeton-methyl		EN 12393			
161	Paclobutrazol					
57	Paraquat					
58	Parathion	EN 1528	EN 12393	CDFA	PDP	
59	Parathion-methyl	EN 1528	EN 12393	CDFA		
182	Penconazole					
120	Permethrin		EN 12393	CDFA	PDP	
128	Phenthoate					
56	2-phenylphenol					

112	Phorate		EN 12393		PDP	
60	Phosalone		EN 12393	CDFA		
102	Phosmet	EN 1528	EN 12393	CDFA		
61	Phosphamidon		EN 12393	CDFA		
141	Phoxim		EN 12393			
210	Pyraclostrobin					
62	Piperonyl butoxide					
101	Pirimicarb					LCMS
86	Pirimiphos-methyl	EN 1528	EN 12393		PDP	
142	Prochloraz					
136	Procymidone		EN 12393	CDFA		
171	Profenofos		EN 12393			
148	Propamocarb					LCMS*
113	Propargite			CDFA		
183	Propham		EN 12393			
160	Propiconazole			CDFA	PDP	
75	Propoxur			CDFA	PDP	LCMS
150	Propylenethiourea (PTU)					
153	Pyrazophos		EN 12393			
63	Pyrethrins		EN 12393			
64	Quintozene		EN 12393	CDFA	PDP	
89	Sec-butylamine					
203	Spinozad					
121	2,4,5,-T					
189	Tebuconazole			CDFA	PDP	LCMS
196	Tebufenozide			CDFA		LCMS
115	Tecnazene		EN 12393	CDFA		
190	Teflubenzuron					LCMS*
167	Terbufos		EN 12393			
65	Thiabendazole			CDFA		LCMS
154	Thiodicarb					LCMS
76	Thiometon		EN 12393			
77	Thiophanate-methyl					
191	Tolclophos-methyl		EN 12393			
162	Tolyfluanid		EN 12393			
133	Triadimefon		EN 12393			
168	Triadimenol				PDP	
143	Triazophos		EN 12393			
66	Trichlorfon		EN 12393			
213	Trifloxystrobin					
116	Triforine					
78	Vamidothion		EN 12393			LCMS*
159	Vinclozolin		EN 12393	CDFA		